THE UNITED STATES PATENT AND TRADEMARK OFFICE

ATTORNEY DOCKET NO. 38137-0019

In re patent application of:
Brust et al.

Divisional of Appln. No.09/131,551 Art Unit: Unassigned

Filed: Herewith – November 29, 2001 Examiner: Unassigned

For: Peptides Derived From A Retrovirus of the HIV Group and Their Use

PRELIMINARY AMENDMENT

Commissioner of Patents
Washington, DC 20231

Sir:

Prior to examination please amend the above-identified Application as set forth in the Amendments below and in the attached Marked-Up Copy. This application is a divisional application of 09/131,551, filed August 10, 1998 which is a divisional application of 08/394,021, filed February 23,1995 which claims priority to German Patent Application No. P4405810.1, filed February 23, 1994.

Amendments

In the Specification:

Page 1, delete the first full paragraph starting with "This application is a divisional of..." and insert the following new paragraph:

This application is a divisional application of 09/131,551, filed August 10, 1998 which is a divisional application of 08/394,021, filed February 23,1995 which claims priority to German Patent Application No. P4405810.1, filed February 23, 1994. Each application is hereby incorporated by reference in its entirety.

Page 4, second full paragraph:

It is therefore an object of the present invention to provide an immunologically active peptide comprising at least 15 consecutive amino acids selected from the amino acids in the following sequence: (SEQ ID NO:1)

Page 6, third full paragraph:

Figure 3 is a diagram showing the sequence region from MVP5180 gp41, expressed in the recombinant plasmid pSEM 41/3-III, in comparison with the corresponding sequence of the HIV-1 isolate ARV-2. (SEQ ID NOS 2-7, 10 and 11 are shown in this Figure.)

Page 11, second full paragraph:

The peptides of the present invention are suitable particular, for the diagnostic detection of antibodies against retroviruses that cause immune deficiency. Such retroviruses are of the HIV type. In a preferred embodiment, these peptides are comprised of a consecutive amino acid sequence of at least 15 amino acids, more preferably of at least 15 to 50, and most preferably of at least 15 to about 35, amino acids selected from the amino acid sequence: (SEQ ID No:1).

Page 11, third full paragraph:

"Consecutive amino acid sequences" are understood by the skilled artisan to mean fragments. In the most preferred embodiment, the peptides comprise consecutive amino acids selected from the sequence (SEQ ID NO:1)
RLQALETLIQNQQRLNLWGXKGKLIXYTSVKWN

Page 11, fifth full paragraph:

If the above amino acid sequence is depicted in the so-called three-letter code, the following sequence is obtained SEQ ID NO:1):

Page 12, second full paragraph:

The present inventors have discovered that an epitope of MVP5180/91, which is of principal relevance for diagnosis is located in the region XKGKLIX (SEQ ID NO:1). Therefore, it is preferable that the peptide of the present invention contain a region having this amino acid sequence.

Page 12, fourth full paragraph

In another embodiment, the peptides according to the invention have a length of about 20 to about 30 amino acids. Within the scope of the present invention, the following peptides are particularly preferred:

MVP601-623 (SEQ ID NO:2): NQQRLNLWGCKGKLICYTSVKWN

MVP591-616C (SEQ ID NO:3) RLQALETLIQNQQRLNLWGCKGKLIC and (SEQ ID NO:4): RLQALETLIQNQQRLNLWGSKGKLIS

Page 20, fourth full paragraph:

The synthesis of MVP 601-623, NQQRLNLWGCKGKLICYTSVKWN (SEQ ID NO:2), as shown in Figure 3, from the transmembrane protein gp41 of MVP5180 was carried out in accordance with Barani, G. and Merrifield, R.B. *in The Peptides, Analysis, Synthesis and* Biology, Vol. 2, Academic Press, Ed. Erhard Gross, Johannes Meyerhofer. Tile analytical purity was 81% according to HPLC. The reference peptide HIV 60L-623, DQQLLGIWGCSGKLICTTAVPWN (SEQ ID NO:5) was likewise synthesized by the Merrifield method. The crude peptide was purified by HPLC. The purity is 87%.

Page 20, fifth full paragraph:

Figure 3 is a diagram showing the sequence region (SEQ ID NO:10) from MVP5180 gp41, expressed in the recombinant plasmid pSEM 41/3-III, in comparison with the corresponding sequence of the HIV-1 isolate ARV-2 (SEQ ID NO:11). The peptides designated HIV are HIV-1 isolatederived sequences (SEQ ID NOS:5-7). The peptides designated MVP are MVP5180-derived sequence (SEQ ID NOS:2-4). The numbering of the sequences relates to the data regarding the HIV-1 BH10 env sequence in Rattner et al., Nature, 313: 277-284

Page 21, second full paragraph:

The peptides MVP 601-623 (SEQ ID NO:2) and HIV 661-623 from Example la were dissolved in 50% (v/v) acetic acid at a concentration of 6 mg/ml. The stock solutions were diluted in 0.10 M sodium bicarbonate (pH 9.6) such that the concentrations of the polypeptides are 1 µg/ml. 100 µl of the dilute solution were added to each of the wells of type B microtitration plates from Nunc, Roskilde, Denmark. The filled test plates were incubated at 2 0°C for 18 hours. The solutions were then sucked off and the wells were rinsed 3-4 times with 300 µl of a 10 g/l solution of bovine serum albumin in phosphate-buffered physiological sodium chloride solution (PBS, pH 7.4), and the test plates were then dried over silica gel at 20°C.

Page 24, second full paragraph:

10 mg of the peptide MVP 601-623 (SEQ ID NO:2) according to the invention (Example 1a) were dissolved in 1 ml of glacial' acetic acid/water (50:50, v/v). When the solution had been neutralized with 5 N sodium hydroxide solution, a 10-fold molar excess of GMBS was added to it and the mixture was incubated at room temperature for 1 hour. The GMBS which had not reacted was separated off by gel filtration (Sephadex G-25) using 0.1 M sodium phosphate/5 mmol/1 nitrilotriacetic acid, pH 6.0. 10 mg of horseradish peroxidase (POD) were incubated, at room temperature for 1 hour, in 5 ml of 10 mmol/1 sodium phosphate, 100 mmol/1 NaCl, pH 8.0), together with a 100-fold molar excess of 2-iminothiolane. Free modifying reagent was then removed by gel chromatography (Sephadex G-25) using 0.1 M sodium phosphate/5 mmol/1 NTA, pH 6.0. The two eluates (SH-activated peroxidase and maleimide-modified HIV-1 peptide) were combined and incubated at room temperature overnight. When the reaction had been stopped using 1/19 vol. of 0.1 M N-ethylmaleimide, the non-reacted HIV-1 peptide was removed from the conjugate by gel chromatography (Sephadex G-25). After the solution has been concentrated (2 mg/ml), the peptide/peroxidase conjugate was stored at -20°C.

Page 26, third full paragraph:

The following 4 peptides were synthesized by the method of

Example 1a:

RILAVERYLKDQQLLGIWGCSGKLIC HIV 591-616 C (SEQ ID NO:6)	Reference
RLLAVERYLKDQQLLGIWGSSGKLIS HIV 591-616 S (SEQ ID NO:7)	peptides
RLQALETLIQNQQRLNLWGCKGKLIC MVP 591-616 C (SEQ ID NO:3)	Peptides
RLQALETLIQNQQRLNLWGSKGKLIS MVP 591-616 S (SEQ ID NO:4)	according to the invention (see Figure 3)

Page 26, seventh full paragraph:

The samples from Examples 1 and 2 were tested, in accordance with Example 3b, in an indirect antibody test both for the peptides MVP 591-616 "C" (SEQ ID NO:3) and MVP 591-616 "S" (SEQ ID NO:4) according -to the invention and for the reference peptides. The results of these investigations are listed in Table 4.

Page 27, fourth full paragraph:

The peptides MVP 601-623 (SEQ ID NO:2) and HIV 601-623 (SEQ ID NO:5); prepared in accordance with Example 1a, were dissolved in 50% (v/v) acetic acid at a concentration of 6 mg/ml. The stock solutions were mixed in different proportions on a volume basis and diluted in 0.10 M sodium carbonate (pH 9.6) such that the total concentration of the peptides is between 0.125 and 2 µg/ml. As in Example 1b, these solutions were added to microtitration plates and the antigens are coated such plates.

Page 28, fifth full paragraph:

1A: 5' TGTGTGGTACCGCAGCGGCAACAGCGCTGACG 3' (SEQ ID NO:8) and
1B: 5' GTGTGTCTAGTTTAGTTATGTCAAACCAATTC 3' (SEQ ID NO:9)

Page 29, third full paragraph:

The expressed MVP5180 sequence is depicted in Figure 3 (SEQ ID NO:10).

In the Claims:

Please cancel claims 1-27 without prejudice or disclaimer and add the following claims:

-- 30. An immunologically active peptide comprising at least 15 consecutive amino acids selected from the amino acids in the following sequence:
VWGIRQLRARLQALETLIQNQQRLNLWGXKGKLIXYTSVKWNTSWSGR,
wherein X is C or S.

31. The peptide of claim 30, wherein said at least 15 consecutive amino acids are selected from the amino acids in the following amino acid sequence:
RLQALETLIQNQQRLNLWGXKGKLIXYTSVKWN
wherein X is C or S.

32. The peptide of claim 30 which binds antibodies against retroviruses of the HIV type.

33. The peptide of claim 30 comprising from 20 to 30 consecutive amino acids.

34. The peptide of claim 30 which further comprises, at one or both ends of the peptide, one or more sequences of amino acids, wherein said sequences are not taken from the amino acid sequence of the retrovirus MVP5180/91.

35. The peptide of claim 30, wherein X is C.

36. The peptide of claim 35, wherein C represents a cysteine residue in an oxidized state.

37. The peptide of claim 35 comprising the amino acid sequence
RLQALETLIQNQQRLNLWGCKGKLIC.

38. The peptide of claim 37, wherein C represents a cysteine residue in an oxidized state.

39. The peptide of claim 35 comprising the amino acid sequence NQQRLNLWGCKGKLICYTSVKNW.

40. The peptide of claim 39, wherein C represents a cysteine residue in an oxidized state.

41. The peptide of claim 30 comprising the amino acid sequence RLQALETLIQNQQRLNLWGSKGKLIS.

42. A diagnostic kit for detecting an antibody against a virus that causes immune deficiency comprising the peptide of claim 30.

43. The kit of claim 42 further comprising at least one control antibody which has a known binding affinity for said peptide.

44. The kit of claim 43 further comprising written instructions for using said kit.

45. A diagnostic composition for detecting in a sample an antibody against a retrovirus that causes immune deficiency, the diagnostic composition comprising the peptide of claim 30 and a detectable label.

46. The diagnostic composition of claim 45, wherein said peptide is detectably labeled.

47. A method of detecting in a sample an antibody against a retrovirus that causes immune deficiency, the method comprising contacting said sample with the diagnostic composition according to claim 45, and detecting the presence of antibody bound to said diagnostic agent as a result of said contacting.

48. An immunogen comprising (a) an amount of the peptide of claim 30 and (b) a physiologically-acceptable excipient therefor, wherein said amount is sufficient to elicit an immune response that protects a susceptible mammal against retrovirus infection.

49. A method for the immunization of a mammal against retrovirus infection, comprising administering to said mammal an effective amount of the immunogen of claim 48.

50. An isolated DNA molecule that encodes the peptide of claim 30.

51. A method of detecting the presence of nucleic acid of a human immunodeficiency virus in a sample, comprising the steps of:

(a) providing a sample suspected of containing one or more nucleic acids encoding a protein of an immunodeficiency virus;

(b) contacting the sample of step a with suitable PCR reagents that comprise at least a first and a second oligonucleotide primer that can anneal to the immunodeficiency virus nucleic acid, wherein the first primer is complementary to a nucleic acid sequence from the MVP5180/91 strain of HIV-1 and the second primer is complementary to a known nucleotide sequence of a protein from an immunodeficiency virus; and

(c) detecting the presence of a geometrially amplified product after incubation under conditions suitable for amplification using both primers.

52. The method of claim 51, wherein the human immunodeficiency virus is HIV-1 or HIV-2.

53. The method of claim 51, wherein the protein of step (b) is selected from the group consisting of the gp41 envelope protein of HIV-1, the p24 protein of HIV-1 or HIV-2, the POL gene of HIV-1 or HIV-2, the LTR gene of HIV-1 or HIV-2, the GAG gene of HIV-1 or HIV-2, the VIF gene of HIV-1 or HIV-2, the ENV gene of HIV-1 or HIV-2, and the NEF gene of HIV-1 or HIV-2.

54. A method of detecting the presence of nucleic acid of a human immunodeficiency virus in a sample, comprising the steps of:

(a) providing a sample suspected of containing one or more nucleic acids encoding a protein of an immunodeficiency virus;

(b) contacting the sample of step a with a labeled DNA wherein the labeled DNA molecule contains a sequence of the MVP5180/91 strain of HIV-1 and the label is capable of generating a signal for detection of the labelled DNA under conditions that allow hybridization of the labeled DNA with complementary nucleic acid to form a hybridization solution; and

(c) detecting a generated signal from the hybridization solution indicating the presence or absence of a nucleic acid encoding the immunodeficiency virus.

55. The method of claim 54, wherein the protein of step (a) is selected from the group consisting of the gp41 envelope protein of HIV-1, the p24 protein of HIV-1 or HIV-2, the POL gene of HIV-1 or HIV-2, the LTR gene of HIV-1 or HIV-2, the GAG gene of HIV-1 or HIV-2, the VIF gene of HIV-1 or HIV-2, the ENV gene of HIV-1 or HIV-2, and the NEF gene of HIV-1 or HIV-2. --

Remarks

Applicant respectfully requests that the foregoing amendments be made prior to examination of the present application. These amendments add no new matter. A first Office Action on the merits is awaited.

The Commissioner is hereby authorized to charge for any excess claim fee, excess independent claims fee and surcharge for late filing to the undersigned's Deposit Account No. 08-1641. In the event any variance exists between the amount authorized and the Patent Office fees, please charge or credit any difference to the undersigned's Deposit Account No. 08-1641.

Please direct all correspondence to the undersigned attorney at the address indicated below.

Respectfully submitted,

Date: December 4, 2001

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26633
PATENT TRADEMARK OFFICE

Marked-Up Version of Specification

Page 4, second full paragraph:

It is therefore an object of the present invention to provide an immunologically active peptide comprising at least 15 consecutive amino acids selected from the amino acids in the following sequence: (SEQ ID NO:1)

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Figure 3 is a diagram showing the sequence region from MVP5180 gp41, expressed in the recombinant plasmid pSEM 41/3-III, in comparison with the corresponding sequence of the HIV-1 isolate ARV-2. (SEQ ID NOS 2-7, 10 and 11 are shown in this Figure.)

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"Consecutive amino acid sequences" are understood by the skilled artisan to mean fragments. In the most preerred embodiment, the peptides comprise consecutive amino acids selected from the sequence SEQ ID NO:1)
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If the above amino acid sequence is depicted in the so-called three-letter code, the following sequence is obtained SEQ ID NO:1):

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In another embodiment, the peptides according to the invention have a length of about 20 to about 30 amino acids. Within the scope of the present invention, the following peptides are particularly preferred:

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Example la:

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RLQALETLIQNQQRLNLWGCKGKLIC MVP 591-616 C (SEQ ID NO:3)	Peptides
RLQALETLIQNQQRLNLWGSKGKLIS MVP 591-616 S (SEQ ID NO:4)	according to the invention (see Figure 3)

Page 26, seventh full paragraph:

The samples from Examples 1 and 2 were tested, in accordance with Example 3b, in an indirect antibody test both for the peptides MVP 591-616 "C" (SEQ ID NO:3) and MVP 591-616 "S" (SEQ ID NO:4) according -to the invention and for the reference peptides. The results of these investigations are listed in Table 4.

Page 27, fourth full paragraph:

The peptides MVP 601-623 (SEQ ID NO:2) and HIV 601-623 (SEQ ID NO:5); prepared in accordance with Example la, were dissolved in 50% (v/v) acetic acid at a concentration of 6 mg/ml. The stock solutions were mixed in different proportions on a volume basis and diluted in 0.10 M sodium carbonate (pH 9.6) such that the total concentration of the peptides is between 0.125 and 2 µg/ml. As in Example 1b, these solutions were added to microtitration plates and the antigens are coated such plates.

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The expressed MVP5180 sequence is depicted in Figure 3 (SEQ ID NO:10).